

CLAIMS

- 1 1. A method for detecting an unbound form of a first member of a binding pair, the
2 binding pair comprising a first and second member, each member bindable to the
3 other, the method comprising the steps of:
 - 4 (a) providing a first particle bound to the second member;
 - 5 (b) reacting the first particle bound to the second
6 member with a sample, thereby forming a first complex between
7 the second member bound to the first particle and unbound first
8 member present in said sample;
 - 9 (c) providing a second particle bound to a third member, the third
10 member being different from the second member and being
11 capable of binding to the first member;
 - 12 (d) reacting the second particle bound to the third
13 member to the sample, thereby forming a second complex between
14 the third member bound to the second particle and the first
15 complex; and
 - 16 (e) detecting any second complex formed.
- 1 2. The method of claim 1, wherein the third member is an antibody which
2 specifically binds to the first member.
- 1 3. The method of claim 1, wherein the first and/or second particle is latex.
- 1 4. The method of claim 1, wherein the second complex is detected by measuring an
2 increase in the turbidity of the sample.
- 1 5. The method of claim 1, wherein steps (a) through (d) are performed sequentially.
- 1 6. The method of claim 1, wherein steps (a) through (d) are performed
2 simultaneously.
- 1 7. The method of claim 1, wherein the amount of second complex formed is
2 quantitated.
- 1 8. The method of claim 1, wherein the first member is protein S.
- 1 9. The method of claim 1, wherein the second member is C4b-binding protein
2 (C4BP).

3 22. The composition of claim 21, wherein the third member binds to the first member
4 at a single binding site which is different from the single binding site to which the
5 second member binds.

1 23. A method for detecting an unbound form of a first member of a binding pair, the
2 binding pair comprising a first and second member, each member bindable to the
3 other, the method comprising the steps of:

4 (a) providing a first particle bound to the second member;

5 (b) reacting the first particle bound to the second member with a
6 sample, thereby forming a first complex between the second
7 member bound to the first particle and unbound first member
8 present in said sample;

9 (c) providing a second particle bound to the first member;

10 (d) reacting the second particle bound to the first member with the
11 sample, thereby forming a second complex between second
12 particle bound to the first member and first particle bound to
13 second member which is not already bound to the first member;
14 and

15 (e) detecting any second complex formed, wherein the amount of
16 second complex formed is inversely proportional to the amount of
17 unbound first member in the sample.

1 24. The method of claim 23, wherein the first and/or second particle is latex.

1 25. The method of claim 23, wherein the second complex is detected by measuring an
2 increase in the turbidity of the sample.

1 26. The method of claim 23, wherein the amount of second complex formed is
2 quantitated.

1 27. The method of claim 23, wherein the first member is protein S.

1 28. The method of claim 23, wherein the second member is C4BP.

1 29. The method of claim 23 wherein the sample is selected from the group consisting
2 of blood, plasma, serum, or an artificially prepared buffer containing the first
3 member.

1 30. A composition for detecting an unbound form of a first member of a binding pair
2 comprising a first and second member, each member bindable to the other, the
3 composition comprising:

4 a first particle bound to the second member; and

5 a second particle bound to the first member.

1 31. The composition of claim 30, wherein the first member is protein S and the
2 second member is C4BP.

1 32. A method for diagnosing thrombophilia comprising performing the method of
2 claim 8, and further comprising comparing the amount of second complex formed
3 to the amount of second complex formed in a sample derived from an individual
4 without thrombophilia.

1 33. A method for diagnosing thrombophilia comprising performing the method of
2 claim 27, and further comprising comparing the amount of second complex
3 formed to the amount of second complex formed in a sample derived from an
4 individual without thrombophilia.
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